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The Evotec BioSystems AG filed a patent application under the title

"Verwendung von Trägermaterial in der Kapillar-Elektro-chromatographie"

on February 3, 2000, with the German Patent and Trade Mark Office.

The attached document is a correct and exact copy of the Original document of this Patent Application.

The application has provisionally received the symbol G 01 N 30/48 of the International Patent Classification with the German Patent and Trade Mark Office.

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Use of Support Materials in Capillary Electrochromatography

The present invention relates to the use of support materials in capillary electrochromatography (CEC).

In general, analytical methods are at best selective; however, only a few, if any, are really specific. Consequently, when an analysis is performed, separation of the analyte from interfering accompanying substances is inevitable.

In chromatographic separations, the sample is dissolved in a mobile phase which may be, for example, a gas, a liquid or a supercritical fluid. The mobile phase is moved through a stationary phase which is not miscible with it and is accommodated in a column, for example, or fixed at a solid surface. The two phases are selected in such a way that the sample components become distributed between the mobile and stationary phases in different ratios. The components which are strongly retained by the stationary phase travel on slowly with the mobile phase. In contrast, the components which are weakly retained by the stationary phase travel fast. Due to these differences, the sample components will separate into discrete bands.

A chromatographic concept which combines the advantages of capillary liquid chromatography (e.g., HPLC) and capillary electrophoresis (CE) is the so-called capillary electrochromatography (CEC). Essentially, CEC can be considered a hybrid of HPLC and CE (Colon et al., Analytical Chemistry News & Features 1995; August 1, 461A-467A). As in HPLC, the components of a sample are separated due to a different distribution between stationary and mobile phases. In addition however, as in CE, an electro-osmotic flow is produced by applying a voltage. The separations can be performed isocratically or with a gradient. The columns are

preferably filled with silica gel particles, typically having particle diameters in a range of from 1 to 5 µm.

An advantage of this method is the possibility of separating anionic, cationic and neutral molecules. However, a great problem lies in the analysis of complex samples, especially biological ones. The latter, such as hemolyzed blood, plasma, serum, milk, saliva, fermenter broth, urine, supernatants of cell culture, food and tissue homogenizates or extracts from natural products, contain a high proportion of matrix components, such as proteins and salts, in addition to the analyte.

Proteins and other macromolecules are precipitated, for example, by high proportions of organic solvents in the mobile phase, or non-specifically and irreversibly bound by residual silanol groups at the surface of a chromatographic support, or denatured. When a porous stationary phase is used, the proteins and other macromolecules block the access to the pores and thus reduce the number of chromatographic adsorption centers. Due to the reduced exchange of materials between the stationary and mobile phases connected therewith, these processes result in a loss of capacity and selectivity of the column. In addition, non-specific adsorption results in variations of the electro-osmotic flow and in non-reproducible retention times of the analytes. In all cases, the CEC column is highly damaged or rendered useless. Therefore, it is necessary to remove these matrix components from the sample prior to the CEC analysis.

These problems are all the more important since they pertain to determinations which are performed in a high number: for example, therapy control, determination of endogenous substances or the high-throughput screening for potential pharmacologically active substances, especially using extracts from natural products.

Common sample processing methods are, for example, cartridge methods or the use of precolumns, preferably filled with silica gel particles, the elution of the analyte preferably being effected by liquid desorption (HPLC).

However, the necessary sample pretreatment steps are often time-, cost- and labor-intensive, and due to the necessary transfer of the analyte to a separating column, result in a volume enlargement of the sample, which results in a loss of selectivity and sensitivity of the separating method.

Boos et al. (LC·GC 1997, 15, 602-611; LC·GC 1996, 14, 554-560) describe a support material based on alkyl-diol silica (ADS) which ensures the quantitative separation of proteins and other macromolecular components. It is characterized by a surface which is inert towards biomolecules, and its pores are occupied by alkyl groups. Its pore size permits small target molecules (analytes) access while the large matrix molecules remain excluded. This material was especially developed for HPLC analyses.

Pinkerton et al. (US Patent 4,544,485) claim a support material for liquid chromatography on a silica or glass basis which enables the separation of proteins or macromolecules from a sample. The so-called internal surface reverse phase (ISRP) material is characterized by a hydrophilic outer surface and a hydrophobic inner or pore surface. In one modification, for example, glycine is bound to the outer particle surface. The pore surface is characterized by polypeptides bound through glycerolpropyl, especially tripeptides. These result in a limited accessibility of the pores. Smaller target molecules (analytes) gain access to the pores while the large matrix molecules remain excluded.

L.J. Glunz et al. (J. of Liquid Chromatography 1992, 15, 1361-1379) also developed a so-called restricted access material based on silica for HPLC, whose functional mechanism relies on a semipermeable membrane (SPS) on the particle surface. Occupation of the surface of the support material with polyethylene glycol or polyoxyethylene produces a network which permits only small analytes access to the pores. Thus, macromolecules are not able to access the pores. The pore surface is occupied by hydrophobic groups, especially phenyl groups, C18, C8 and nitrile.

The methods of capillary electrochromatography and HPLC are considerably distinct, in particular, by the electro-osmotic forces occurring in CEC. Thus,

materials and conditions suitable for HPLC cannot be simply transferred to the CEC method (Colon et al., Analytical Chemistry & Features 1997; August 1).

Therefore, it was all the more surprising that the use of support materials characterized by having a surface which consists of regions of different derivatization and/or functionality, in capillary electrochromatography enables an essentially quantitative separation of the analyte from other sample components, especially proteins and other macromolecular components (sample matrix) of the sample.

The term "derivatization" relates to the covalent or, in particular, adsorptive binding of molecules to the surface of the support material. This may be, for example, synthetic or natural polymers which, as a chemical diffusion barrier, prevent macromolecules of the sample matrix from adsorbing to or denaturing on the support material. The term "functionalization" refers to the properties of a respective region, in particular. Thus, particular regions of the support material can be hydrophobic while other regions have hydrophilic properties. Such a functionalization can be achieved by a different derivatization of the regions. Thus, different molecules (e.g., fatty acids in one region, alcohols in another) can be employed. However, it is also possible to achieve a different functionalization by varying the coverage density of regions with identical molecules.

The use of the support material in CEC according to the invention permits to separate the analyte from other components of the sample without diluting it. In connection with isotachophoresis, in one embodiment, it is possible to transfer the analyte onto a separating column, especially another CEC column or a µ-HPLC column, without significantly increasing the volume. Even sample volumes of $\leq$ 10 µl can be processed.

In the use of the support material according to the invention, the reproducibility with respect to plate numbers, retention time and resolution of the column is retained even after the repeated injection of complex samples, especially samples containing serum and cell culture media.

In another embodiment, the use of the support material according to the invention even permits the combined sample processing and separation of complex samples on a single CEC column. With respect to the separating performance, sensitivity, signal-to-noise ratio, selectivity, service life of the column and costs, it is equivalent or even superior to sample processing and separation performed on separate columns. This for the first time enables the use of such a system in a high-throughput process, such as the high-throughput screening for potential pharmacologically active substances.

It may be preferred to pass the analytes separated by CEC, preferably in a fully automatic manner, to another analysis, especially using fluorescence correlation spectroscopy, during which the interaction of the analyte with other molecules is detected, in particular. This can be, for example, receptor-ligand interactions.

Thus, the use of the support material according to the invention is altogether characterized by the following properties:

- there is a possibility of repeated direct injection of untreated samples, especially biological samples, on one CEC column;

- the protein matrix is quantitatively removed;

- the analyte can be concentrated at the upper brim of the column and quantitatively separated off and into its components independently of the matrix;

- high separating performance, sensitivity, accuracy, very good signal-to-noise ratio;

- high extent of reproducibility with respect to plate numbers, retention time and resolution of separation in the column;

- automatic operation is possible;

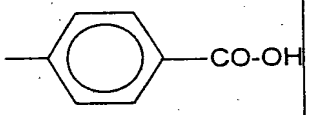
- high number of analytical runs, continuous operation of the column;

- low costs per analysis.

Particularly good separation results are obtained if the support material is porous, that is, the surface thereof consists of an outer surface an a pore surface.

It is particularly advantageous if the surface of the support material is derivatized and/or functionalized with hydrophobic and/or hydrophilic groups and/or ion-exchange groups and/or affinity ligands. Thus, the support material can be designed individually with respect to its chemical and/or physical separating properties.

The functional groups listed in the following are particularly suitable:

Hydrophilic groups	Hydrophobic groups	Ion-exchange groups	Affinity ligands
- amides; - alcohols; - nitriles; - nitroalkyl; - polyethers	- alkyl, particularly preferably C1-C40; - aryl, in particular - phenyl; - benzyl; - halides; - SH; - esters, in particular carboxylic acid esters; - alkoxy; - ketones	$-(CH_2)_xSO_3H$; $-(CF_2)_xSO_3H$; $-(CH_2)_x-{}^+NR_3{}^-OH$; $-(CF_2)_x-{}^+NR_3{}^-OH$; $-(CH_2)_x-CO_2H$; $-(CF_2)_x-CO_2H$;  R = -alkyl, -H; x = 0-30	- antibodies; - Fab fragments; - molecular imprinted polymer (MIP); -proteins; - receptors; - DNA; - oligonucleotides

For the separation of antigens from serum, it is, for example, preferred, to use support materials which are functionalized by polyclonal antibodies on the pore surface thereof and have an outer surface which is rendered hydrophilic, e.g., by diol groups.

The separation of the reaction product from synthetic mixtures containing hydrophilic polymers, in particular polyethylene glycol, and hydrophilic reactants such as, e.g., oligonucleotides, may preferably be performed by using support materials with the following properties: the pore surface is derivatized by diols or sugars. The outer surface thereof has hydrophobic properties due to a derivatization by C8 alkyl residues.

In the purification of antisense oligonucleotides from serum it is advantageous to use support materials having a hydrophilic outer surface, which preferably is obtained by a derivatization by alcohols, and a pore surface which is derivatized by anion exchangers, preferably $-NR_3^+ \ ^-OH$, with R = -ethyl, -propyl.

According to the invention, the use of support materials having regions with different derivatizations and/or functionalizations which are homogeneously or heterogenously distributed over the surface is preferred in CEC. Thus, for example, it may be desired to use a support material having regions on the surface thereof which contain a functional group in varying densities.

As the starting material, e.g., silicate-containing materials, especially porous silicate-containing materials can be employed. As described in DE 4130475, said materials can be hydrophilized with hydroxyl groups on their outer surfaces, whereas the pores and hence the inner surface are occupied by fatty acid esters.

In particular, porous particles from silica gel or glass modified with 2,3-dihydroxypropoxy groups may be used. Said particles may also consist of an organic polymer or copolymer containing hydroxy groups.

Also suitable as the starting material are hydrophilic organic copolymers of, for example, oligoethylene glycol, glycidyl methacrylate or pentaerythritol dimethacrylate. They can be functionalized by acrylamide derivatives of the formula CH_2=CH-CO-NHR, R being, for example, a linear and/or branched-chain aliphatic sulfonic acid group and/or carboxylic acid group.

It is particularly preferred to use a support material whose surface has regions derivatized and/or functionalized with alkyl residues having a length of C1 to C50, preferably C4 to C22, more preferably C4, C8 and C18. In particular, the alkyl residues are on the inner surface of the support material, i.e., within the pores.

It is also advantageous to use a support material whose surface has regions derivatized and/or functionalized with diols.

Also preferred is the use of a support material which has a substantially spherical design, particularly good separation results being achieved by the use of support materials having an outer diameter, D, of $0.05 \leq D \leq 20$ µm, preferably $0.1 \leq D \leq 5$ µm. Thus, for example, support materials having a size of $0.5 \leq D \leq 3$ µm can be employed.

If the support material has a porous design, it is advantageous for it to have a pore diameter, d, of $0.5 \leq d \leq 100$ nm, preferably $1 \leq d \leq 50$ nm, more preferably $2 \leq d \leq 6$ nm.

It is advantageous to use the support material according to the invention in a CEC method for sample processing, wherein the sample consisting of an analyte and other sample components

- is applied to a CEC column system;
- an electro-osmotic flow is produced by applying a voltage, whereby the sample molecules are moved and/or the sample molecules migrate according to their charge-to-mass ratio;
- the sample matrix is eluted by applying a washing buffer;
- the analyte is eluted by applying a transfer buffer.

Especially preferred is the use according to the invention in a CEC method for the combined sample processing and separation, wherein the sample consisting of an analyte and other sample components

- is applied to a CEC column system;

- an electro-osmotic flow is produced by applying a voltage, whereby the sample molecules are moved and/or the sample molecules migrate according to their charge-to-mass ratio;
- the sample matrix is eluted by applying a washing buffer;
- the analyte is separated and eluted by applying an elution buffer.

The application of the sample to the column and the quantitative removal of the matrix is preferably performed hydrodynamically and/or electro-osmotically and/or electrophoretically, which results in a concentration of the analyte by a factor of from 10 to 1000.

However, it may also be preferred to apply the sample to the column and wash it in a flash-back method. This reduces a possible contamination of the column with matrix components and also results in concentration of the sample at the top of the column.

It is also advantageous to perform the elution and separation of the analytes hydrodynamically and/or electro-osmotically and/or electrophoretically.

Particularly preferred is the electro-osmotic elution and separation of the analytes in order to obtain a sufficient plate number even when very short columns, preferably of $\leq$ 10 cm, are used.

In another embodiment, a further concentration of the analyte by a factor of from 10 to 1000 is achieved by isotachophoresis during the elution. Thus, for example, the analyte can be transferred from a separate sample processing column to a separating column without a substantial change in volume.

For increasing the selectivity of the method and/or for increasing the electro-osmotic flow, mixtures of different support materials are preferably employed as the stationary phase.

A particular advantage of such mixtures is their permitting optimum adaptation to the properties of the mobile phase and the sample and thus ensuring a sensitive and selective separation.

The mobile phase is preferably composed of buffer salts in the presence of anionic and/or cationic and/or zwitterionic ion pairing reagents; the composition thereof may directly be adjusted to the nature and the properties of the analyte to obtain a good separating performance, as, e.g., the examples demonstrate. However, with unknown compositions it is also possible to use so-called universal buffers optimized for the separation and elution of analytes having unknown compositions.

To achieve especially good separation results, it is also desirable for the washing buffer and the eluting buffer to contain organic solvents; then, the washing buffer should contain at least 1% and eluting buffer at least 20% of an organic solvent.

In all, in a particularly preferred embodiment of the process the stationary and the mobile phases should be selected such that the electro-osmotic flow remains constant during the binding and elution, respectively, of the analyte or changes in a reproducible manner with a electro-osmotic flow in the range of from 0.5 to 10 mm/s being preferred. Preferably, a direct current high voltage is used for the generation of the electro-osmotic flow.

If a detection of the analytes is to be effected subsequent to the separation and elution, it is desirable, especially in mass-spectrometric detection, for the buffer salts and/or anionic and/or cationic and/or zwitterionic ion-pair reagents to be volatile at room temperature.

For an accurate characterization of the composition of the analyte, both qualitatively and quantitatively, it is possible, in a preferred embodiment, to perform various spectrometric and spectroscopic analytical methods subsequent to the separation and/or elution. Of particular advantage are the methods of mass spectrometry and/or optical detection, for example, by light scattering, especially condensation nucleation light scattering detection (Szostek et al., 1997, Analytical

Chemistry, 69, 2955-2962), and/or electrochemical detection. Thus, for example, UV detection is employed in Example 1.

However, it may also be preferred to supply the analyte fractions to a fraction collector subsequent to the separation, i.e., collect them individually to be passed to a further use.

In another embodiment, transfer to another column system may also be desirable for further separation.

Of particular advantage is the possibility of a parallel operation of the method in a multitude of interconnected CEC column systems.

CEC devices which are suitable for the use according to the invention are known to the skilled person.

In another preferred embodiment of the device, the sample receiving means is designed to receive samples having a volume, V, of 0.5 nl $\leq$ V $\leq$ 100 µl. It is of particular advantage to use CEC columns having a length of from 0.1 to 100 cm and a diameter of $\leq$ 500 µm.

Further embodiments of the device are explained below with reference to the enclosed Figures.

Figure 1 shows a CEC column system which consists of a single column for the sample processing and/or separation.

Figure 2 shows the coupling of a CEC column system to a detector, in this case a mass spectrometer.

Figure 3 shows the coupling of a CEC column system to another column.

Figure 4 shows the coupling of a CEC column system to a fraction collector.

Figure 5 shows a CEC chip system.

Figure 6 shows a possible embodiment of a CEC chip system which is coupled to a capillary system.

Figure 7 shows in an illustrative way a possible embodiment of a µ-total analysis system.

Figure 8 shows the electropherogram of an analyte mixture, separated on a CEC column packed with ISPR GFFII-S5-80.

Figure 9 shows the electropherogram of an analyte mixture, separated on a CEC column packed with SPS 5PM-S5-100-phenyl.

Figure 10 shows in an illustrative way a particle of a porous support material.

A particularly advantageous embodiment of the device is represented in Figure 1. A CEC column (30) packed with the support material (60) according to the invention is immersed with both ends in the container (90) with the mobile phase (120). The voltage source (10) serves for applying a voltage between the two ends of the columns. The voltage enables the build-up of an electro-osmotic flow in the column. In addition, a device for applying pressure to the containers may also be provided. The applying of pressure uniformly to both ends of the column counteracts the degassing of the buffer solutions and thus the formation of air bubbles in the column. One column end is designed for taking up the sample. A changing device enables the changing of the containers (90) and thus the changing or adaptation of the buffer solutions (120) to the process step. By providing, for example, a detector (150) as outlined in Figure 1 directly on the column, the analyte can be directly detected and analyzed.

In a further embodiment of the device, it is preferred that the column system consist of at least one CEC column for sample processing and at least one CEC column for separation of the analyte which are interconnected through a capillary

system, wherein this capillary system, in a particularly preferred embodiment, has at least one outlet through which the sample matrix can be removed.

In addition, it is also possible to use a CEC column (30) only for sample processing. A possible embodiment thereof is represented in Figure 3. This example shows the combination of a CEC column (30) with another column (170) arranged on a chip. It is also possible to transfer the analyte to other analytical or separating systems after separating off the sample matrix.

The device may also comprise a coupling of the column system to at least one detector, especially a mass spectrometer and/or light-scattering detector or other optical detector, and/or electrochemical detector (150). This preferred embodiment is outlined in Figure 2 with coupling to a mass spectrometer which comprises an electrospray device (230). For coupling to a detector, it may be preferred for the outlet of the column system to have an inner and/or outer diameter which is different from that of the inlet.

In addition, in another preferred embodiment of the device, it is provided that the column system (30) may be coupled to a fraction collector and/or another column system. This may be effected, for example, by direct coupling. In a preferred embodiment (Figure 4), for fraction collection, a voltage is applied between the inlet of the column and, for example, a gold-coated MALDI plate (200) (Meeting Abstract, Advances in Mass Spectrometry, January 7-8, 1999, Orlando, Florida, USA). The eluate is atomized, and the analyte is selectively collected in individual wells of the plate.

In another advantageous embodiment, the CEC column system is arranged on a chip (300) (Figure 5). A column system is represented which consists of a CEC column (30) with a support material (60) according to the invention, a sample reservoir (290) and three buffer reservoirs (260). The system is designed in such a way that each reservoir can accommodate one electrode. Thus, a voltage can be selectively applied between different reservoirs. In a preferred embodiment, the addressing and switching of the electrodes is effected automatically, wherein the exact circuit diagrams can be managed by computer programs.

In a particularly advantageous embodiment, the chip system (300) is combined with glass capillaries or CEC columns (30) made of fused silica. An example of this embodiment of the device according to the invention is outlined in Figure 6.

For preparing the capillaries and chip systems, it is preferred to use materials such as plastics, glass, fused silica, ceramics, elastomers or polymers.

The preparation of suitable chips can be effected, for example, by applying photolithography in connection with etching techniques. This has been described, for example, by J.P. Landers (Handbook of Capillary Electrophoresis, 1997, CRC Press, page 828) for the preparation of chips for use in capillary electrophoresis. Materials such as glass or fused silica are coated with a photosensitive substance. The desired channel system is transferred to the substrate by exposure to light using a mask and etched into the substrate, for example, in a bath of diluted HF/NH_4F. For substrates of fused silica, it is necessary to apply a gold/chromium thin film to the substrate as an etching mask.

Depending on the material employed for the preparation of the capillaries for the CEC columns and chip systems, it may be desirable to coat the capillary interior surface to prevent non-specific reactions of the sample with, for example, free silanol groups thereon. This is advantageously effected with PVA or polyacrylamide.

In another particular embodiment of the device, the column system is a component of a total analysis system.

A possible total analysis system is represented in Figure 7. Such a system represents the entirety of an analytical system and can equally comprise the sample processing and analysis and optionally upstream and/or downstream steps. The µTAS represented in Figure 7 comprises the labeling of a protein with a dye, the separation of the dye and of the unlabeled protein through a CEC column (30) packed with the support material (60) according to the invention, and the detection of the labeled protein. The system outlined here can be integrated, for

example, on a chip, the round recesses being capable of respectively accommodating electrodes for applying a voltage.

Another embodiment provides for the parallel operation of a multitude of CEC column systems in which the sample processing and separation is performed in parallel. These column systems are chip systems or capillary systems or combinations of both. Such a coupling of several systems is advantageous, in particular, in high-throughput screening since it allows the parallel processing and separation of a multitude of samples, which can then be further examined.

Figure 8 shows the electropherogram of an analyte mixture in a model matrix. The performance was effected with a CEC column filled with ISRP GFFII-S5-80 (pore size 8 nm). Length of the packed capillary: 8.3 cm. Inner diameter: 100 µm. Detection wavelength: 210 nm. Further conditions, see Example 1.

Figure 9 shows the electropherogram of an analyte mixture in a model matrix. The performance was effected with a CEC column filled with SPS 5PM-S5-100-phenyl (pore size 10 nm). Length of the packed capillary: 8.3 cm. Inner diameter: 100 µm. Detection wavelength: 210 nm. Further conditions, see Example 2.

Figure 10 shows in an illustrative way a particle of a porous support material. The surface of said support material may be divided into an outer surface (510) and a pore surface (540).

Example 1:

Separation of a mixture of analytes in a model matrix

Materials employed:

The CEC column having a length of 8.3 cm and an inner diameter of 100 µm was packed with Pinkerton ISRP GFFII-S5-80 supplied by Regis® Technologies, Inc., Austin, USA. The particles employed had a diameter of 5 µm and a pore size of 8 nm.

The washing buffer consisted of 5% of acetonitrile, 95% of water, 5 mM ammonium acetate (pH 8.5). The elution buffer consisted of 40% of acetonitrile, 60% of water, 5 mM ammonium acetate (pH 8.5).

The solution contained FBS (fetal bovine serum) in a concentration of 10 mg/ml, and 1 mg/ml each of thiourea, acetaminophen, benzocain, propranolol and quinine. In the following, this sample solution is referred to as "mixture of analytes in a model matrix".

Device

For performing the separation of the mixture of analytes in a model matrix, the device shown in Figure 1 was employed. The column packed with ISPR GFFII-S5-80 was immersed with its ends each in a container for receiving buffer solution. Using a voltage source (10), a voltage was applied between the two ends of the column.

Column preparation

The column preparation was performed at 15 °C in 2 steps:

1. The column was first equilibrated with separating buffer. During this process, the voltage was increased stepwise in steps of 5 kV from –5 kV up to –20 kV at intervals of 5 min while a pressure of 5 bar was applied to the inlet buffer container (buffer container into which the inlet of the column is immersed). Then, a pressure of 10 bar was applied to both buffer containers, and a voltage of –15 kV was applied. The stability of the column was monitored in the meantime by measuring the current and the UV absorption (210 nm).

2. The 2nd equilibration phase was performed in washing buffer and took 12 min, a voltage of –15 kV and a pressure of 10 bar being applied to both buffer containers. The current and the voltage were also monitored.

After the end of the 2nd phase, the current and UV absorption were stable.

Separations

During the whole operation, the temperature of the buffers, the samples and the separating capillary was controlled to 15 °C.

Mixture of analytes in a model matrix

The sample (mixture of analytes in a model matrix) was electrokinetically charged onto the column by applying a voltage of –5 kV for 3 seconds. Subsequently, a small amount of washing buffer, a so-called buffer plug, was charged onto the column under the same conditions in order to prevent the possible diffusion of the sample into the buffer container.

Subsequently, the sample was washed by applying the washing buffer at a voltage of –15 kV and applying a pressure of 10 bar to both ends of the column to remove the proteins and salts of the model matrix from the CEC column. After 3 minutes, the washing buffer was replaced by an elution buffer. The conditions of –15 kV and 10 bar were retained. Thiourea eluted at 1.48 min, acetaminophen at 2.27 min, benzocain at 4.88 min, propranolol at 4.99 min, and quinine at 5.67 min. The electropherogram of this separation is shown in Figure 8.

Example 2:

Separation of a mixture of analytes in a model matrix

Materials employed:

The CEC column having a length of 8.3 cm and an inner diameter of 100 µm was packed with SPS 5PM-S5-100-phenyl supplied by Regis® Technologies, Inc., Austin, USA. The particles employed had a diameter of 5 µm and a pore size of 10 nm.

The washing buffer consisted of 5% of acetonitrile, 95% of water, 5 mM ammonium acetate (pH 8.5). The elution buffer consisted of 15% of acetonitrile, 85% of water, 5 mM ammonium acetate (pH 4.7).

The solution contained FBS (fetal bovine serum) in a concentration of 10 mg/ml, and 1 mg/ml each of thiourea, acetaminophen, benzocain, propranolol and quinine. In the following, this sample solution is referred to as "mixture of analytes in a model matrix".

Device

For performing the separation of the mixture of analytes in a model matrix, the device shown in Figure 1 was employed. The column packed with SPS 5PM-S5-100-phenyl was immersed with its ends each in a container for receiving buffer solution. Using a voltage source (10), a voltage was applied between the two ends of the column.

Column preparation

The column preparation was performed in accordance with Example 3.

Separations

During the whole operation, the temperature of the buffers, the samples and the separating capillary was controlled to 15 °C.

The sample (mixture of analytes in a model matrix) was electrokinetically charged onto the column by applying a voltage of –5 kV for 3 seconds. Subsequently, a small amount of washing buffer, a so-called buffer plug, was charged onto the column under the same conditions in order to prevent the possible diffusion of the sample into the buffer container.

Subsequently, the sample was washed by applying the washing buffer at a voltage of –15 kV and applying a pressure of 10 bar to both ends of the column to remove the proteins and salts of the model matrix. After 6 minutes, the washing buffer was replaced by an elution buffer. The conditions of –15 kV and 10 bar were retained. Thiourea eluted at 1.94 min, acetaminophen at 4.23 min, propranolol and

quinine at 11.34 min, and benzocain at 18.25 min. The electropherogram of this separation is shown in Figure 9.

CLAIMS

1. Use of support material in capillary electrochromatography (CEC), characterized in that said support material has a surface having regions of different derivatization and/or functionality.

2. The use of support material according to claim 1, characterized in that said support material has a porous design and the surface consists of an outer surface (510) and a pore surface (540).

3. The use of support material according to claim 1 and/or claim 2, characterized in that said surface is derivatized and/or functionalized with hydrophobic and/or hydrophilic groups and/or ion-exchange groups and/or affinity ligands.

4. The use of support material according to at least one of claims 1 to 3, characterized in that said regions of different derivatization and/or functionality are distributed on said surface homogeneously and/or heterogeneously.

5. The use of support material according to at least one of claims 1 to 4, characterized in that said surface comprises regions derivatized and/or functionalized with alkyl residues having a length of C_1 to C_{50}, preferably C_4 to C_{22}, more preferably C_4, C_8 and C_{18}.

6. The use of support material according to at least one of claims 1 to 5, characterized in that said surface has regions derivatized and/or functionalized with diols.

7. The use of support material according to at least one of claims 1 to 6, characterized in that said support material has a substantially spherical design having an outer diameter, D, of $0.05 \leq D \leq 20$ µm, preferably $1 \leq D \leq 5$ µm, more preferably $0.5 \leq D \leq 3$ µm.

8. The use of support material according to at least one of claims 1 to 7, characterized in that said support material has a pore diameter, d, of $0.5 \leq d \leq 100$ nm, preferably $1 \leq d \leq 50$ nm, more preferably $2 \leq d \leq 6$ nm.

9. The use of support material according to at least one of claims 1 to 8, characterized in that said support material consists of hydroxy-containing materials with reverse phases, wherein said reverse phases are restricted to the pore surfaces and consist of fatty acid esters.

10. The use of support material according to at least one of claims 1 to 9, characterized in that said support material consists of silica gel modified with 2,3-dihydroxypropoxy groups.

11. The use of support material according to at least one of claims 1 to 10, characterized in that said support material consists of glass modified with 2,3-dihydroxypropoxy groups.

12. The use of support material according to at least one of claims 1 to 11, characterized in that said support material consists of a hydroxy group-containing organic polymer or copolymer.

13. The use of support material according to at least one of claims 1 to 12, characterized in that said support material consists of silicate-containing material modified with polyethylene glycol or polyoxyethylene on its outer surface, and in that the pore surface is modified with hydrophobic groups, especially phenyl groups, C_{18}, C_8 and/or nitrile.

14. The use of support material according to at least one of claims 1 to 13, characterized in that said support material consists of hydroxy-containing material modified with glycine on its outer surface and modified with polypeptides, especially tripeptides, on the pore surface.

15. The use of support material according to at least one of claims 1 to 14, characterized in that said support material consists of silica gel modified with glycerolpropyl.

16. The use of support material according to at least one of claims 1 to 15, characterized in that said support material consists of glass modified with glycerolpropyl.

Abstract

The invention pertains to the use of a support material in capillary electrochromatography (CEC), characterized in that said support material has a surface which consists of regions of different derivatization and/or functionality.

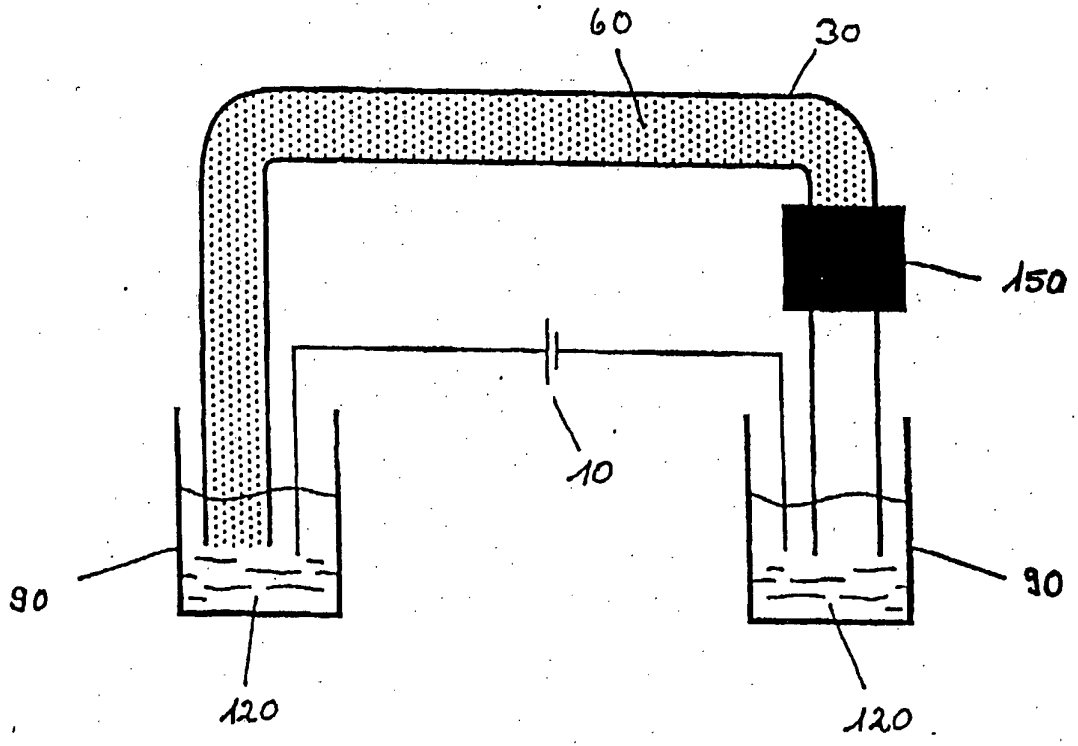


Fig. 1

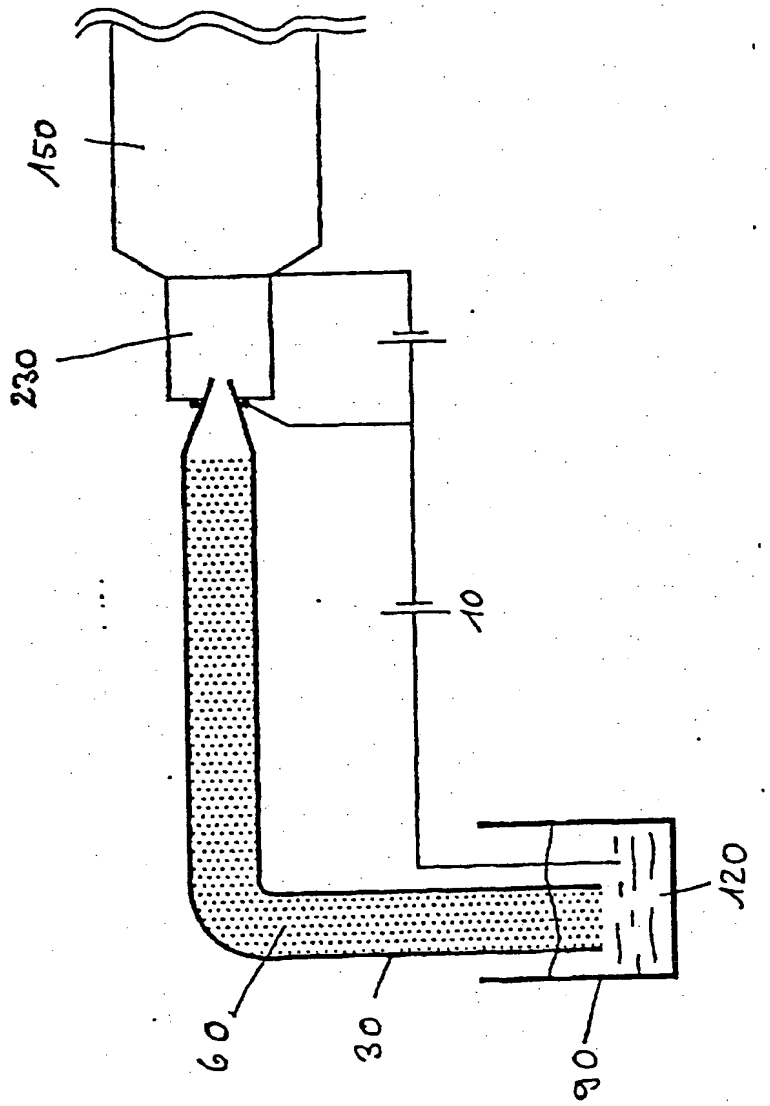


Fig. 2

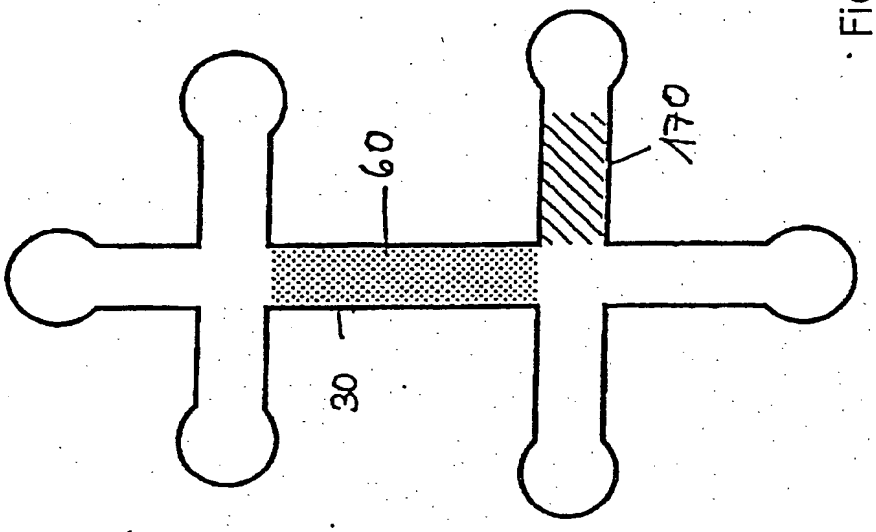
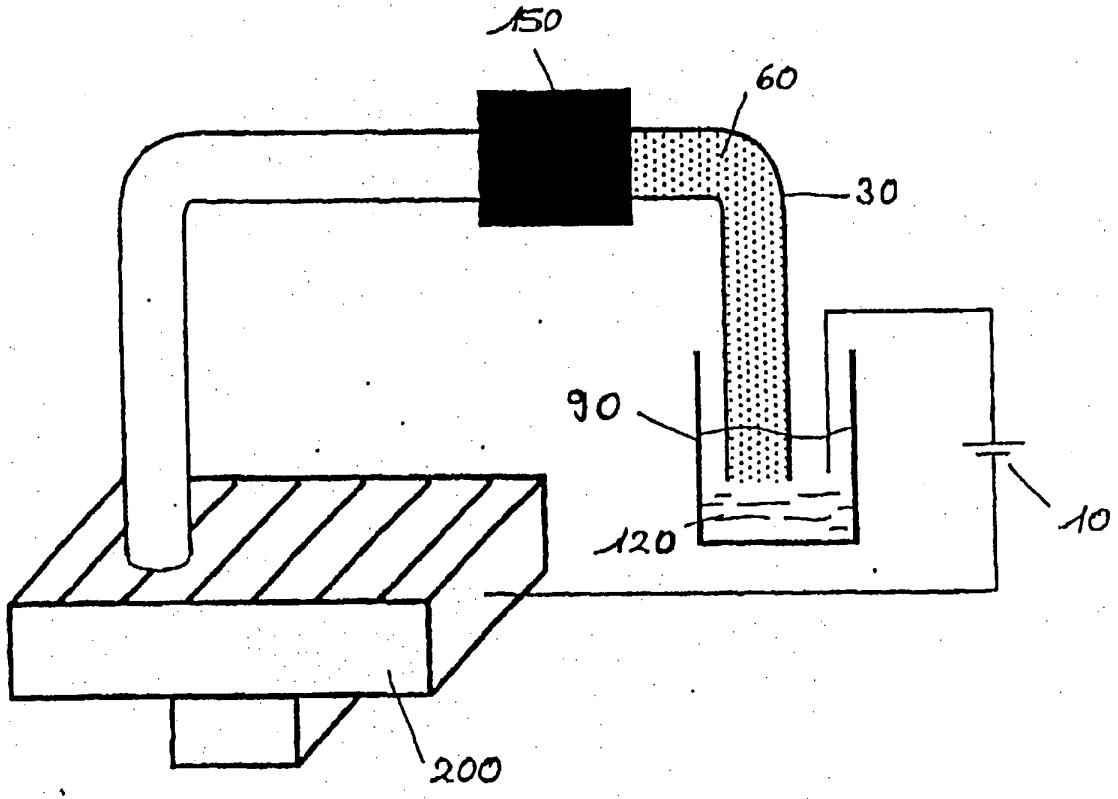


Fig. 3

Fig. 4



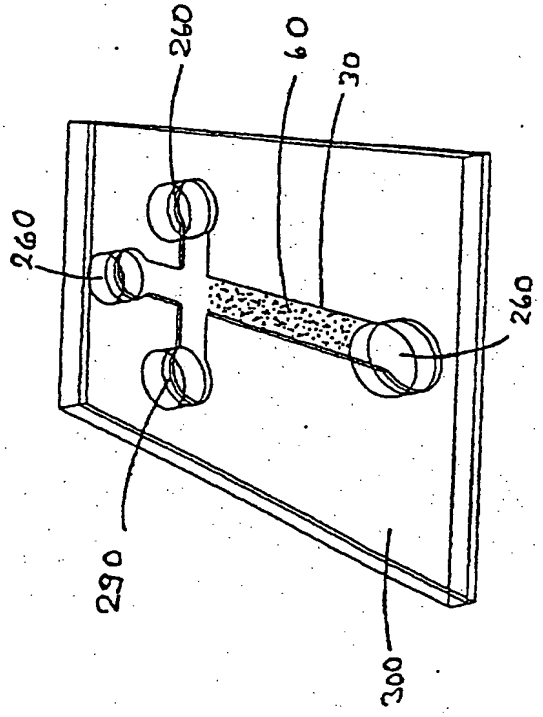


Fig. 5

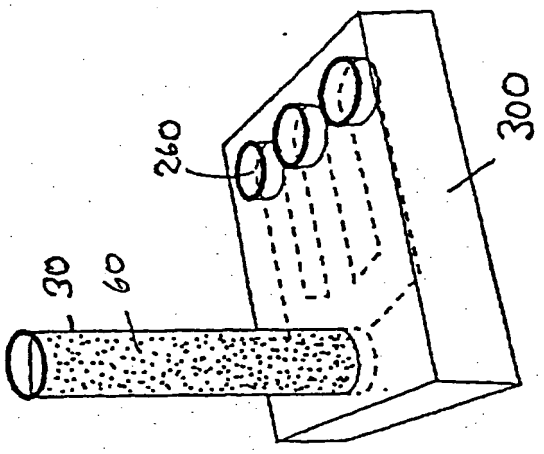


Fig. 6

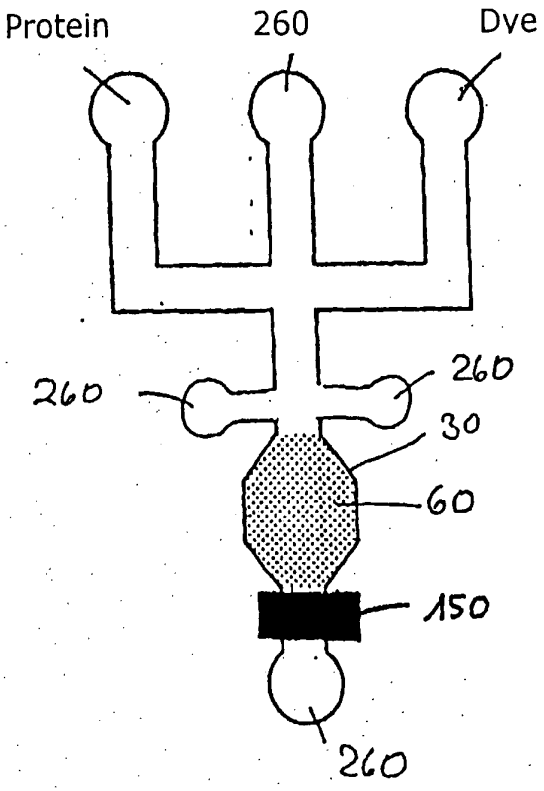
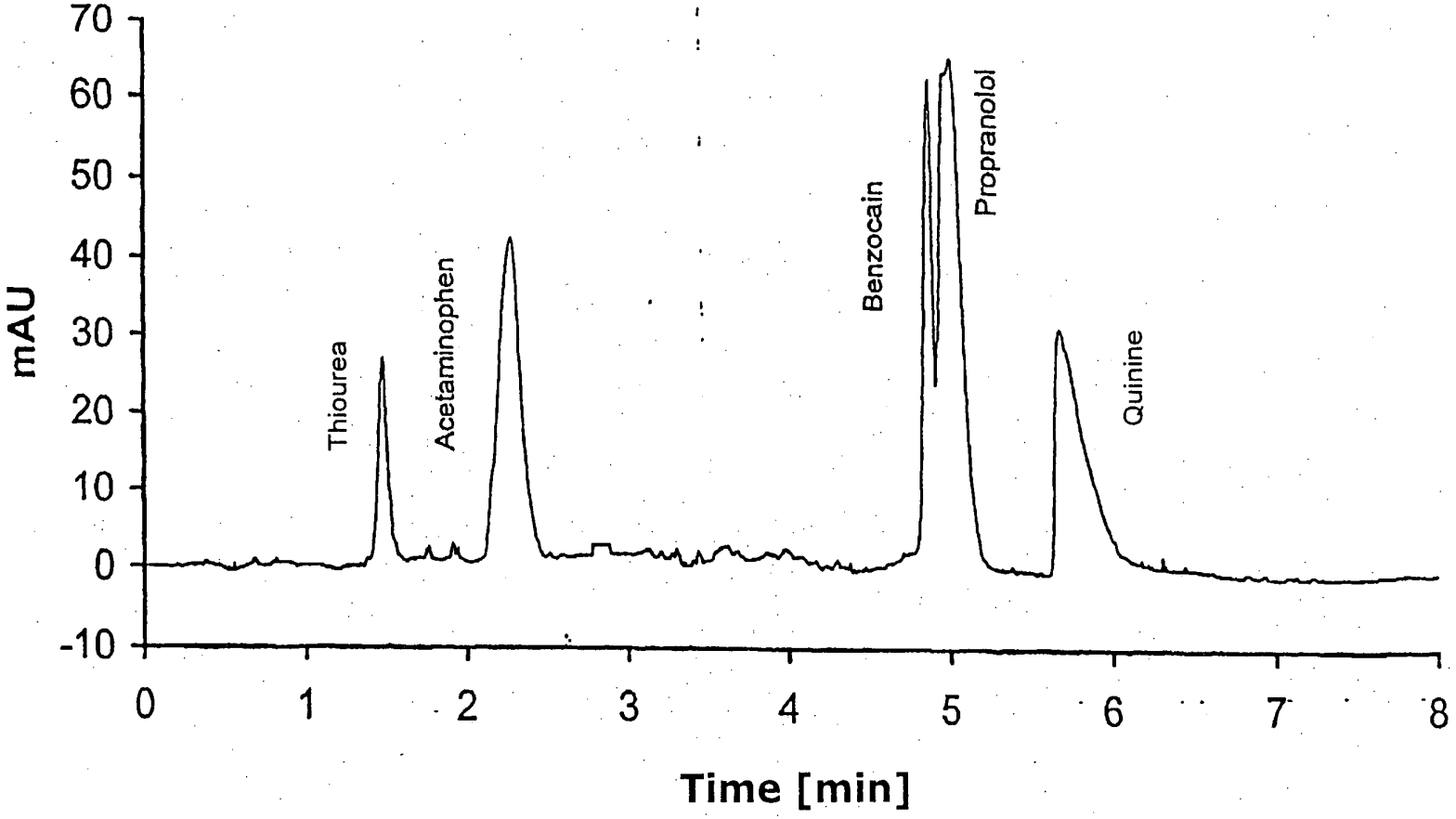


Fig. 7

Fig. 8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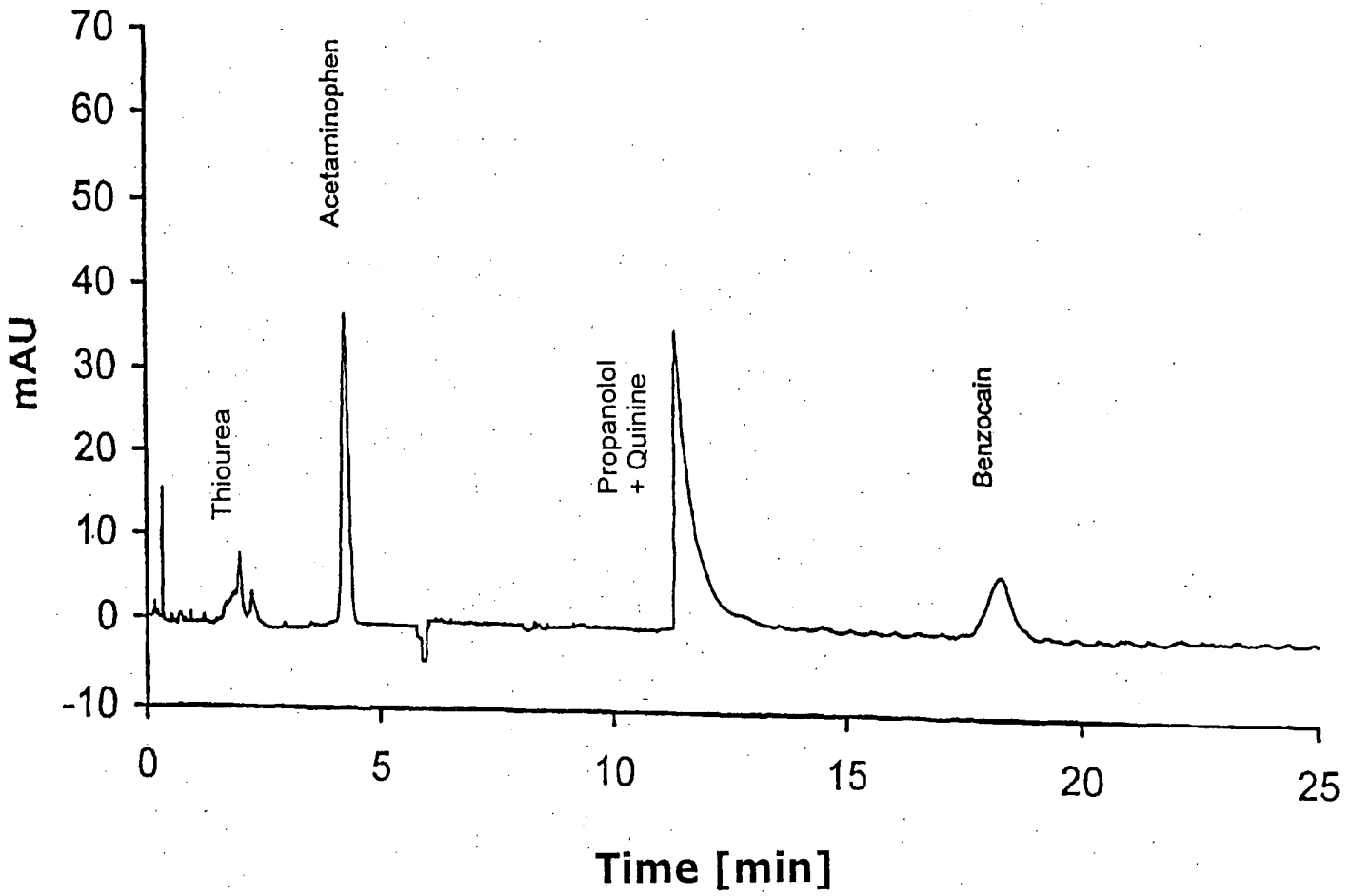
60
50
40
30
20
10
0
-10
mAU
0
1
2
3
4
5
6
7
8
Time [min]
Thiourea
Acetaminophen
Benzocain
Propranolol
Quinine

Fig. 9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60
50
40
30
20
10
0
-10
mAU
Thiourea
Acetaminophen
Propanolol
+ Quinine
Benzocain
0
5
10
15
20
25
Time [min]

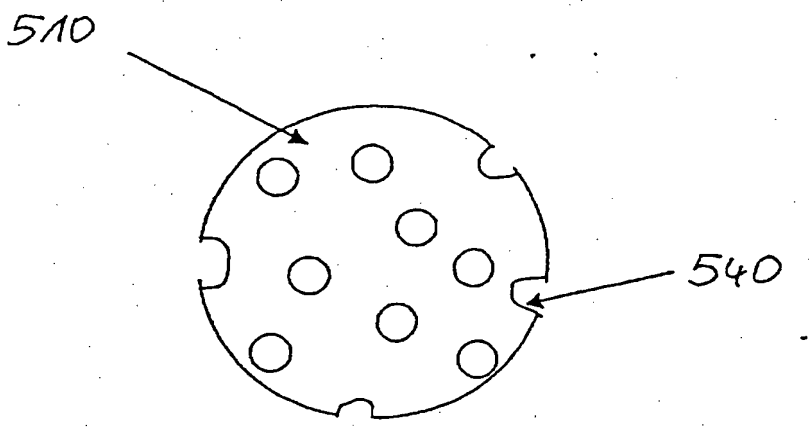

Fig. 10